MOLECULAR SYSTEMATICS AND THE CONTROL OF INVASIVE PLANTS: A CASE STUDY OF *TAMARIX* (TAMARICACEAE)[1]

John F. Gaskin[2]

ABSTRACT

The invasion of habitats by non-native organisms is considered, behind habitat destruction, the second largest threat to biodiversity worldwide. Consequently, control of invasive organisms is now an integral part of ecosystem stewardship. Effective control may require explicit characterization of the invasion at the family, species, and/or population levels. *Tamarix* is considered one of the worst plant invasions in the United States. A synopsis of molecular systematic advances at the family and species levels is presented, and the impact on the control of *Tamarix* is discussed. Also, a preliminary population-level analysis of *T. ramosissima* is performed using chloroplast DNA sequence data. This analysis investigates origins of invasive haplotypes and tests for the presence of cultivated haplotypes in the invasion. Advances in understanding invasions through molecular systematic and population-level studies will prove to be powerful tools in many control scenarios.

Key words: biodiversity, biological control, conservation, invasion, molecular systematics, population structure, phylogeny, tamarisk, *Tamarix*, saltcedar.

The invasion of habitats by non-native organisms is considered the second largest threat to biodiversity worldwide behind habitat destruction (Wilson, 1997). In the United States exotic plants now represent 17.3% of the flora (Kartesz & Meacham, 1999), and approximately 400 of the 972 plants and animals listed by the Endangered Species Act are at risk primarily due to competition with and predation by non-native species (Stein & Flack, 1996). For these reasons, the control of invasives is becoming an integral part of ecosystem stewardship.

Methods of controlling invasive plants include manual removal, fire, herbicides, biological control, and legislation of import and sale. Effective control of invasive plants often requires explicit characterization of the invasion at the family, species, and/or population levels.

Several species of the genus *Tamarix* L. (common name: saltcedar or tamarisk, family Tamaricaceae) are, as a group, considered one of the worst plant invasions in the southwestern U.S. (TNC, 2002). This invasion is the subject of localized manual, chemical, and legislative control efforts and a large-scale biological control project conducted by the United States Department of Agriculture. Additional legislative control may be required, as cultivars of *Tamarix* are still available from numerous horticultural suppliers. The effective implementation of biological control projects of *Tamarix* (e.g., DeLoach et al., 2000) has been influenced by phylogenetic concerns at the following levels:

(1) FAMILY LEVEL

Phylogenetic relationships of the invasive plant's family are important when biological control is proposed. Control agents must be tested for their risk of host-switching by confronting the control agent with plant species from closely related plant families. In the past, Tamaricaceae usually were placed in the plant order Violales of the Dilleniidae (e.g., Cronquist, 1981), but recent molecular sequence data analyses have altered the traditional ordinal placement of many plant families, and Tamaricaceae are now included in the Caryophyllales (APG, 1998). These changes will alter the plant taxa to be

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[2] Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, U.S.A. Present address: USDA-ARS-NPARL, P.O. Box 463, Sidney, Montana 59270, U.S.A. jgaskin@sidney.ars.usda.gov.

Table 1. Putative U.S. *Tamarix* invasives complied from McClintock (1951), Baum (1967), and Crins (1989).

Putative U.S. invasive	Taxonomic and morphological notes
T. africana Poir.	morphologically similar to *T. canariensis* and *T. gallica* in aestival floral form (Baum, 1978)
T. aralensis Bunge	rarely cultivated, not extensively naturalized (Baum, 1967)
T. aphylla (L.) H. Karst.	morphologically dissimilar to all other U.S. *Tamarix*
T. canariensis Willd.	morphologically similar to *T. gallica* (Crins, 1989)
T. chinensis Lour.	morphologically similar to *T. ramosissima* (Crins, 1989)
T. gallica L.	morphologically similar to *T. canariensis* (Crins, 1989)
T. juniperina Bunge	synonym of *T. chinensis* (Baum, 1978)
T. parviflora D.C.	morphologically dissimilar to all other invasive U.S. *Tamarix*
T. pentandra Pall.	synonym of *T. ramosissima* (Baum, 1978)
T. ramosissima Ledeb.	morphologically similar to *T. chinensis* (Crins, 1989)
T. tetrandra Pall.	U.S. invasive specimens with this name considered to be *T. parviflora* (Baum, 1967)
T. tetragyna Ehrenb.	naturalized in eastern U.S., not yet invasive (Crins, 1989)

tested in the risk analysis of biological control agent host-switches.

(2) Species Level

The *Tamarix* invasion consists of many species, some of which are morphologically very similar. The specific identities of taxa involved in the invasion are controversial (Crins, 1989), in part, because most *Tamarix* species cannot be distinguished without flowers or fruit present. Precise species identity is needed to determine the geographic origin of the invasive species and its coevolved biological control agents. *Tamarix* is one of the more taxonomically challenging genera among the angiosperms (Baum, 1978), and intermediate states exist for some morphological characters used in species identification. These character states can often vary on a single individual from season to season (Rusanov, 1949), and hybridization may play a role in the taxonomic confusion (Rusanov, 1949; Wilken, 1993). Improper species identification could lead to searches for biological control agents perhaps outside the native range of the invasive plant. The failure of morphological data to elucidate the identities of invasive *Tamarix* species necessitates the use of molecular data as an additional source of taxonomic information.

(3) Population Level

Population-level investigation of any of the invasive *Tamarix* may be necessary if the biological control agents are extremely host-specific, and if the invasive plant species has widespread origins. Initial biological control tests show that imported insects have differential effectiveness on what putatively appears to be a single species of *Tamarix* (*T. ramosissima* Ledeb.) collected from different regions of the U.S. (DeLoach & Tracy, 1997), raising the issue of infraspecific geographical variation. Many species of *Tamarix* are widespread in Eurasia (Baum, 1978), and it is unlikely that much of the genetic diversity of any one species was imported to the United States. Historical records do not reveal precise origins or genetic information concerning the introductions (Horton, 1964). The control agents being tested (e.g., saltcedar leaf beetle, *Diorhabda elongata*) may not have evolved with the invasive, and thus might result in ineffective or sub-optimal control. For these reasons, it would be useful to know how many genotypes are represented in the U.S. invasion, and to what degree we can pinpoint their Eurasian origins.

Additionally, *Tamarix* is still being horticulturally distributed in the United States. Policy makers need to determine if the genotypes currently being promoted predominate in the invasive populations. Based on their similar morphology, invasive *Tamarix* is often indistinguishable from cultivars. Molecular evidence of contemporary cultivars contributing to the *Tamarix* invasion could greatly influence future policy decisions regarding the sale and distribution of these plants.

Background

Tamarix is an Old World genus of approximately 54 species (Baum, 1978). Eight to twelve of these (Table 1) were imported to the United States from southern Europe or Asia in the 1800s to be used for shade and erosion control (Baum, 1967), and an aggressive subset has overtaken more than 1,000,000 riparian acres (Brotherson & Field, 1987). This infestation is expanding by 40,000 acres per year (DiTomaso, 1998), eroding the biodiversity of many western U.S. natural areas, including major river systems and national parks.

Tamarix species initially invade by germinating

during wet periods or in riparian areas. Once established, they can tolerate drought by utilizing deep groundwater sources. They also exude excess salt from salinized water sources from glands in their scale-like leaves (Neill, 1985), which are seasonally dropped, forming a thick saline duff on the soil surface that inhibits the germination of other plants. In the U.S., *Tamarix* species are avoided by most avian frugivores and insectivores (Brotherson & Field, 1987), and only two mammal species (the desert wood rat and desert cottontail) are known to feed upon them, with minimal damage to the plants (DiTomaso, 1998). *Tamarix* invasions lower biodiversity levels by displacing typical Southwestern riparian vegetation such as cottonwood and willow (Hughes, 1993), as well as the insects, birds, and mammals that these native trees support (Neill, 1985). Their profuse growth alters stream and river dynamics by narrowing channel width (Robinson, 1965), and invasions can extend over 1 km on each side of a river (e.g., Gila River, southwestern Arizona, and Colorado River south of Blythe, California, U.S.; J. Gaskin, pers. obs.). Dense stands of *Tamarix*, with their high rates of transpiration, can substantially lower the water table, and have caused perennial springs and creeks to dry up, in some cases threatening regionally rare or federally listed species such as the desert pupfish and the desert slender salamander (Kerpez & Smith, 1987).

Tamarix invasions have proven difficult to control. These plants cannot be killed easily by fire, by cutting at ground level, or by herbicide applied to the foliage alone. Effective removal is both expensive and potentially damaging to the habitat, requiring mechanical uprooting, or cutting at ground level with application of a systemic herbicide to the stump. Repeated treatments are often necessary (Neill, 1985). Control is possible on a small scale, but land managers are often forced to live with large invasions due to prohibitive control costs (Stein & Flack, 1996). For these reasons biological agents were proposed as an alternative means of control.

Well-researched biological control projects often come under heavy public scrutiny due to the potentially dire effects of control agent host-switches (Thomas & Willis, 1998). Therefore, biological control researchers must unambiguously know the identity of the invasive *Tamarix* and its relationships to native species. Improper taxonomic identification may lead to searches for control agents outside the native range of the invasive species and thus wasted efforts or less-effective biocontrol agents. Improper identification of the invasive could also lead to the collection of biological control agents that have historic ties to sympatric congener plant species or to genotypes with a different phenology or developmental timing, again yielding ineffective biological control. Considering that the average biological control research program spans many years at a cost of hundreds of thousands to millions of dollars (Gillot, 1995), it is logical and economical to predicate a biological control project with precise taxonomic knowledge of the invasive plant.

Knowing the genotype of an invasive plant is especially important when choosing a fungal, bacterial, or viral control agent involved in a gene-for-gene resistance/virulence interaction (Kerr, 1987). Even insects are often species-specific, and in some cases, host-specificity can reach to the level of the plant genotype. An example is the differential herbivory of the Hessian fly (*Mayetiola destructor*) on different genotypes of wheat (*Triticum aestivum* L.) (Schoonhoven et al., 1998). Also, differential herbivory on plant populations has been detected in willow trees (*Salix*) under natural conditions (Rank, 1991).

The saltcedar leaf beetle (*Diorhabda elongata*) from western China is already being investigated as a potential *Tamarix* control agent in quarantined and field releases (C. J. DeLoach, pers. comm.). In no-choice tests, newly hatched *D. elongata* larvae were placed in vials, each with leaf material from a different plant specimen. The plants were collected from different areas of the United States and grown in common garden plots. Using morphology, all specimens were determined to be the same species (*T. ramosissima*). Observations on the feeding and life span of the insects were recorded, and survival of the insects to adulthood on different plant specimens varied from 34% to 0% (DeLoach & Tracy, 1997). The reduced survival on several of the *T. ramosissima* plants may have in part been caused by less than optimal physiological condition of some of the plants (DeLoach & Tracy, 1997), but genotypic differences in the plants also may have influenced the results.

The search for *Tamarix* biological control agents continues, as the Agricultural Research Service of the United States Department of Agriculture does not expect that the current control agents will achieve satisfactory control of saltcedar in all areas, and perhaps as many as 8 to 12 additional insects as specific herbivores will be required (DeLoach & Tracy, 1997). This is based on biological control of other invasive plants, such as cacti, lantana, and leafy spurge, which have required up to 15 or more insect species introductions (DeLoach & Tracy, 1997) for effective control.

Cultivars

Tamarix ramosissima is commonly sold today as an ornamental plant. Cultivars of *T. ramosissima* include 'Pink Cascade', 'Rosea', 'Rubra', and 'Summer Glow'. The most common of these is *T. ramosissima* 'Pink Cascade', known for its dense, dark pink plumes of flowers (due to mostly compound, not simple, inflorescence racemes) and finely textured bluish foliage. These cultivars and invasive populations are almost identical in floral and vegetative structures, and may only differ in the intensity of flower color, density of inflorescences, and foliage color. Invasive *T. ramosissima* is highly variable in flower color within some populations, ranging from deep red to white. On a single invasive plant, both simple and compound racemes can be found, making the density of inflorescences also highly variable. Invasive foliage color can vary within populations from dark green to the blue-gray found in the *T. ramosissima* 'Pink Cascade' cultivar (J. Gaskin, pers. obs.).

As a weedy species, the *Tamarix* cultivars are easy to grow and tolerant of poor soils. They are available through many nurseries, catalogs, and from internet sales (e.g., Gertens Online Shop, www.gertens.com). *Tamarix ramosissima* is not legally available in Colorado, Nevada, Washington, and Wyoming, where it is listed as a noxious weed (USDA, 2002).

Molecular Systematics and the Control of *Tamarix*

family level

Biological control agents are assessed for their risk of host-switching by placing them on U.S. native plants that are closely related to *Tamarix*. Tamaricaceae, along with the sister family Frankeniaceae, had historically been placed in the order Violales (Cronquist, 1981). Therefore, U.S.D.A. researchers tested if the *Tamarix* control agents would feed and reproduce on plants from other families in this order, such as Frankeniaceae and Fouquieriaceae (DeLoach & Tracy, 1997). Recent DNA sequence data analyses strongly suggest that Tamaricaceae and Frankeniaceae actually belong together in the order Caryophyllales (APG, 1998), closely aligned with families such as Droseraceae and Polygonaceae (Lledó et al., 1998). This phylogenetic rearrangement requires a substantially different set of test plants in the greenhouse, which may provide significantly different assessments of the risk of host-switching.

species level

A recent study used DNA sequence data to determine how many invasive *Tamarix* species were naturalized in the United States and to see if the molecular data were congruent with the morphological distinctions currently used to segregate taxa (Gaskin & Schaal, in press). The taxonomy and morphology of the 12 putative U.S. naturalized *Tamarix* species were investigated (Table 1). Three of the species names had been designated as synonyms, and two were not yet considered invasive, leaving seven putative invasive taxa. A molecular phylogenetic analysis of these and other selected species in the genus was performed from samples collected in the western U.S., Argentina, and wild native populations across Eurasia and southern Africa (voucher information is listed in Appendix 1).

Phylogenies from both nuclear ribosomal ITS and chloroplast *trn*S-*trn*G intergenic spacer sequence data were constructed and compared. Portions of the final phylogenies presented in Figure 1 illustrate incongruence with earlier taxonomic understanding of the genus. For example, note that *T. chinensis* Lour. and *T. ramosissima*, thought to belong in different sections of the genus (sects. *Oligadenia* and *Tamarix*, respectively), have identical placement on both phylogenies. Additionally, the most recent sectional classification of the genus (Baum, 1978) was not significantly similar to either the chloroplast or nuclear topologies found in Gaskin and Schaal (in press).

For many samples there was incongruence between the chloroplast and nuclear evolutionary histories. For example, in the nuclear phylogeny of Figure 1, *T. ramosissima* specimen *Schulte 1* was in a clade with all of the other *T. ramosissima*, but in the chloroplast phylogeny it appeared in a clade with *T. canariensis* Willd. (*Gaskin 3049*) and *T. gallica* L. (*Gaskin 3039*). Similarly, a *T. canariensis* specimen (*Kirk 2*) was in a chloroplast clade with another *T. canariensis* (*Gaskin 3020*), but in the nuclear phylogeny it was found far from specimen *Gaskin 3020*, as the sister to the *T. ramosissima* clade. These incongruences of chloroplast and nuclear evolutionary histories, which were significant based on the Templeton test (Templeton, 1983), supported a hypothesis of hybridization (Whittemore & Schaal, 1991; Soltis & Kuzoff, 1995).

The study concluded that morphology within *Tamarix* is often misleading as a means of identifying specimens. Also, though not all putative invasive species could be distinguished with molecular data, there was enough phylogenetic resolution to recognize four invasive *Tamarix* entities in the U.S.:

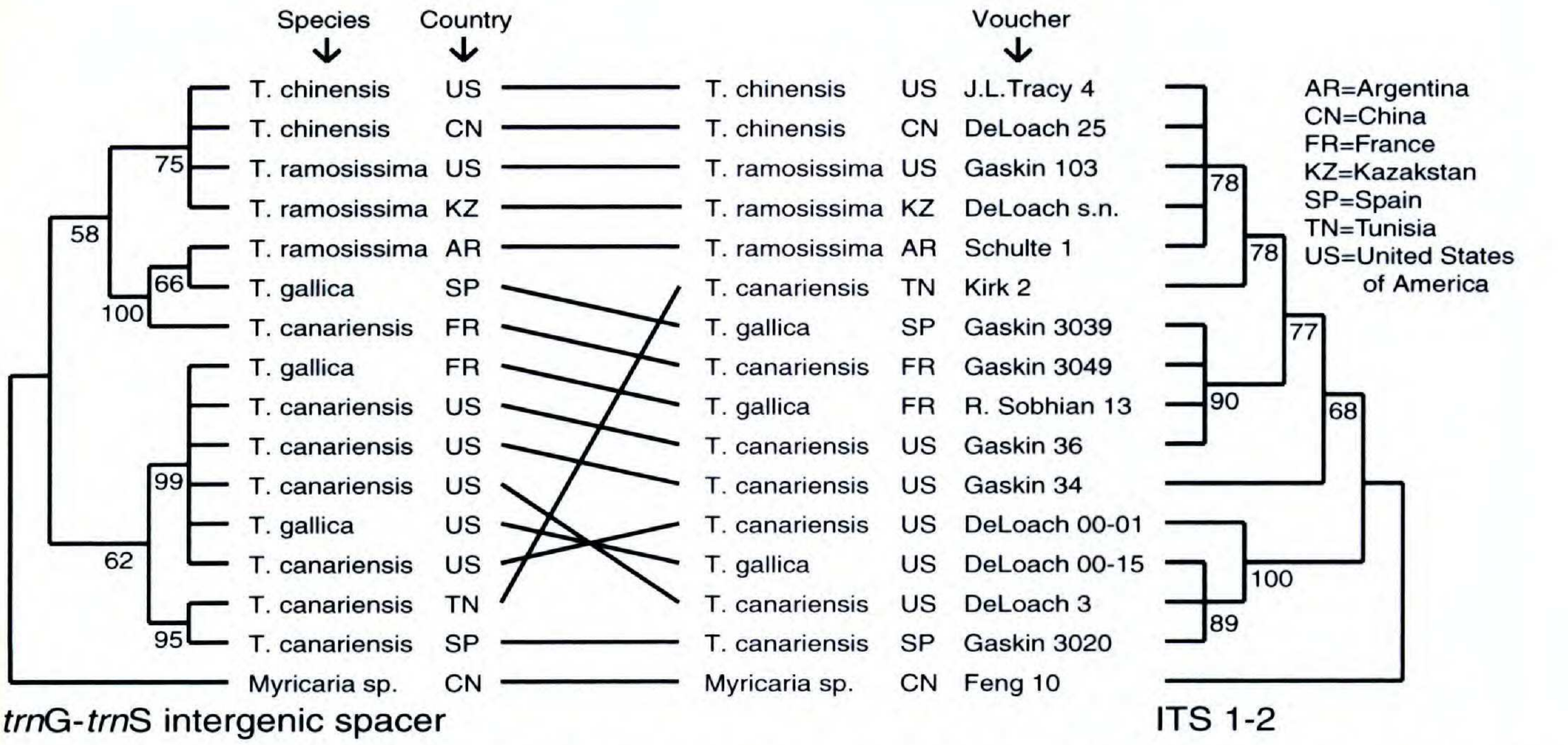

Figure 1. Chloroplast and nuclear marker phylogenies. On the left is the single most parsimonious tree for the chloroplast sequence marker (*trn*S–*trn*G intergenic spacer), 218 steps in length, with a C.I. of 0.99 and an R.C. of 0.95. On the right is the strict consensus of the 30 most parsimonious trees for the nuclear sequence marker (ITS1–2), 179 steps in length, with a C.I. of 0.95 and a R.C. of 0.88. Numbers below lines are bootstrap values. The same specimens were used in each analysis, and are connected by lines in between the two phylogenies. Adapted from Gaskin and Schaal (in press).

(1) *T. aphylla* (L.) H. Karst, (2) *T. parviflora* DC., (3) *T. canariensis*/*T. gallica*, and (4) *T. chinensis*/*T. ramosissima*. Additionally, there was evidence of introgression between *T. ramosissima*, *T. canariensis*, and *T. gallica*, which is a likely source of confusion in the characterization of some *Tamarix* invasions (Gaskin & Schaal, in press).

POPULATION LEVEL

To examine the Eurasian origins and relationships of *T. chinensis* and *T. ramosissima* invasive genotypes, and to investigate the presence of cultivated haplotypes in the invasion, the highly variable 1001 bp chloroplast *trn*S-*trn*G intergenic spacer is analyzed using the primers of Hamilton (1999). A gene tree, which infers genealogical relationships of DNA sequence haplotypes (alleles), is constructed to represent the populations and their relationships (see Fig. 2).

A total of 59 cultivated, invasive, and native *T. ramosissima* or *T. chinensis* specimens was collected, with 33 samples from the New World and 26 from the Old World. The identities of most specimens were determined using Baum's (1978) morphological descriptions and keys. Voucher information is listed in Appendix 1.

In the chloroplast sequence aligned data set, 93 (9.3%) of the sites are variable. There are 12 (1.2%) single bp changes, three single base insertion/deletions, one 2-bp indel, and three prominent indels that vary from 8 to 55 bp in length. All indels are treated as a single event (a fifth base). A most parsimonious gene tree (or minimum spanning network) of 22 steps was assembled by hand, representing the fewest mutations that explain the relationships of the specimens (Fig. 2).

The molecular analysis presents population-level information that is unobtainable using morphology alone. For example, the *T. ramosissima* species is represented by a total of seven haplotypes, marked A through G, on the gene tree (Fig. 2). The specimens and their origins are also presented in the boxes. The lines separating the gene tree boxes represent single point mutations or indel events. The small circles represent inferred intermediate haplotypes that may be extinct, may not have been collected during sampling, or may not have ever existed if mutations did not accumulate in single steps. Interesting results include the following:

(1) Of the seven haplotypes found, four are represented in the western U.S. *Tamarix* invasion. Haplotype A is very common, representing 46 (78%) of the specimens sampled. The native haplotype A specimens were collected in the Republic of Georgia, Iran, Turkmenistan, Kazakstan, China, and South Korea. The naturalized U.S. specimens were collected from California, east to Texas, north to Kansas, and west to Washington. The widespread nature of this haplotype will not facilitate pinpointing its invasive origins in Eurasia. Finer resolution

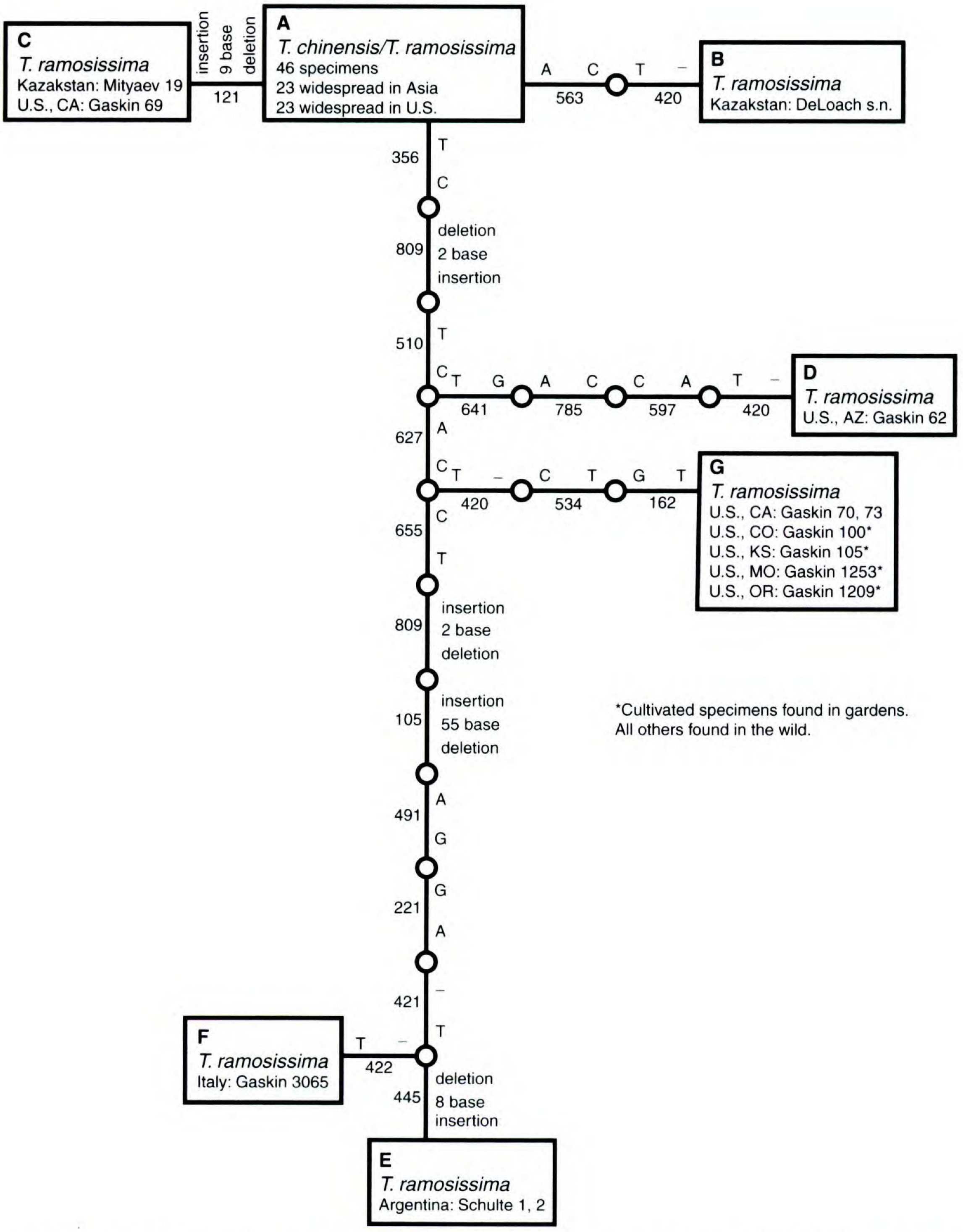

Figure 2. Single most parsimonious gene genealogy of the chloroplast sequence marker *trn*S–*trn*G intergenic spacer for *T. ramosissima* and morphologically similar species. The gene tree is 22 steps (mutations) in length. The haplotype (allele) designation is in each box, along with information on the number and distribution of specimens with that haplotype. The lines separating the haplotype boxes are single point mutations or insertion/deletion events. The small circles represent intermediate haplotypes not recovered in this analysis. The gene tree is interpreted in the following manner: Haplotype A differs from haplotype B by two mutations. One of these is a single nucleotide mutation at site #563 along the *trn*S–*trn*G intergenic spacer, where haplotype A has an adenine (A) and haplotype B has a cytosine (C). The other difference is at site #420, where haplotype A has a thymine (T), and haplotype B has lost this thymine in a deletion event (-).

markers are needed to distinguish if there is unrevealed population structure or if the haplotype A plants are genetically similar across Eurasia. If these plants are genetically similar across the native range, collection of insects from any area of Eurasia will be equally likely to find control agents that have evolved with this haplotype.

(2) Haplotype C is rarer than A, representing only two of the specimens (Fig. 2), and was found once in southern California and once in Kazakstan. This haplotype is only one mutation different from the common A haplotype, but that mutation is a prominent 9 bp indel event that was not found in any other samples. This presents evidence that at least a small part of the invasion may have its origins in Kazakstan.

(3) Haplotype D was found once, in Arizona. The plant containing this haplotype (*Gaskin 62*) morphologically resembled *T. ramosissima*. In a different study, this haplotype was found to be common in another species, *T. parviflora* (Gaskin & Schaal, in press). *Tamarix parviflora* is an invasive species with tetramerous floral structure, morphologically very distinct from the pentamerous floral structure of *T. ramosissima* and *T. chinensis*. This incongruence between morphology and haplotype may be due to hybridization, as was found in the genus-wide study (Gaskin & Schaal, in press).

(4) Haplotype E was found twice in Argentina invasions by *Tamarix*, but never in the U.S. This haplotype is genetically quite distinct from the common A haplotype, differing by 11 mutations, including two notable 8 and 55 bp indel events. This genotype has not been found in Eurasia, indicating that further sampling of native *Tamarix* populations is needed.

(5) All cultivated U.S. specimens of *Tamarix* contain haplotype G. This haplotype was not recovered in Eurasian sampling. Haplotype G differs from the common haplotype A by at least seven mutation events. The haplotype G, as representative of cultivar introgression, was found once, as an invasive, near the Salton Sea in California (*Gaskin 70*). Even though the presence of this genotype is not common in the invasion, its ability to invade is now confirmed. Any presence of cultivar haplotypes in invasions should serve as a strong forewarning in future policy decisions regarding the horticultural use of invasive taxa.

The preceding chloroplast sequence marker data allow us to begin to delve into the genetic structure of the *T. ramosissima/T. chinensis* invasion. This preliminary analysis is of a small sample size, and a more in-depth population analysis is in preparation, using highly variable nuclear DNA sequence markers such as phosphoenolpyruvate carboxylase introns. I plan to continue sequencing selected *Tamarix* that exhibit resistance to biological control agents. If they are determined to be genotypically distinct from the susceptible *Tamarix*, their Eurasian origins will be provided to the biological control exploration project. Knowing the number of haplotypes that comprise a plant invasion, their origins, and the ability of cultivars to contribute to the invasion are powerful tools to document and control problematic exotic plant species.

Conclusion

Molecular analyses will have an increasing role in invasive plant control efforts. At the family level they will enable more accurate risk assessments of biological control host-switching. At the species level, molecular systematics will help elucidate invasive species identities and any morphologically cryptic hybridization events. At the population level, molecular systematics will allow the unprecedented characterization of invasive taxa as genotypes, allowing precise matching of biological control agents with their targets, and elucidating links between cultivars and invasions of plants. These advances in understanding plant invasions will enhance control efforts and contribute to the protection of native biodiversity.

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Internet Resources

TNC (The Nature Conservancy). 2002. ⟨http://tncweeds.ucdavis.edu/worst.html⟩.
USDA (United States Department of Agriculture). 2002. ⟨http://www.aphis.usda.gov/npb/statenw.html⟩.

Appendix 1. Vouchers for exemplars used in DNA sequencing, and corresponding GenBank accession numbers. (*) = USDA-ARS Grassland Soil and Water Research Lab, Temple, Texas, U.S.A. OW = Old World, NW = New World.

trn S-*trn*G haplotype	Species	Origin	Collection #	DNA specimen #	GenBank accession *trn* S–*trn*G	GenBank accession ITS 1–2
A	*Tamarix* cf. *ramosissima* Ledeb.	OW: China	*DeLoach s.n.* (*)	164	AF490798	
A	*Tamarix chinensis* Lour.	OW: China	*DeLoach 00-13* (*)	0.13	AF490798	
A	*Tamarix chinensis* Lour.	OW: China	*DeLoach 25* (*)	23	AF490798	AF484770
A	*Tamarix chinensis* Lour.	OW: China	*DeLoach s.n.* (*)	140	AF490798	
A	*Tamarix chinensis* Lour.	OW: China	*USDA 00-45* (*)	2011	AF490798	
A	*Tamarix chinensis* Lour.	OW: S. Korea	*Gaskin 202* (MO)	202	AF490798	
A	*Tamarix chinensis* Lour.	NW: U.S., TX	*DeLoach 00-14* (*)	0.14	AF490798	
A	*Tamarix chinensis* Lour.	NW: U.S., TX	*J. L. Tracy 4* (*)	22	AF490798	AF484776
A	*Tamarix* cf. *ramosissima* Ledeb.	OW: China	*DeLoach s.n.* (*)	141	AF490798	
A	*Tamarix* cf. *ramosissima* Ledeb.	OW: Turkmenistan	*Gaskin 1107* (MO)	1107	AF490798	
A	*Tamarix ramosissima* Ledeb.	OW: Georgia	*Gaskin 229* (MO)	292	AF490798	
A	*Tamarix ramosissima* Ledeb.	OW: Georgia	*Gaskin 505* (MO)	310	AF490798	
A	*Tamarix ramosissima* Ledeb.	OW: Georgia	*Gaskin 508* (MO)	315	AF490798	
A	*Tamarix ramosissima* Ledeb.	OW: Kazakstan	*I. D. Mityaev 20* (*)	33	AF490798	
A	*Tamarix ramosissima* Ledeb.	OW: Kazakstan	*V. Ivlev s.n.* (MO)	419	AF490798	
A	*Tamarix ramosissima* Ledeb.	OW: Kazakstan	*V. Ivlev s.n.* (MO)	422	AF490798	
A	*Tamarix ramosissima* Ledeb.	OW: Kazakstan	*V. Ivlev s.n.* (MO)	423	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., KS	*DeLoach 00-21* (*)	0.21	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., NM	*DeLoach 00-23* (*)	0.23	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., CA	*DeLoach 00-24* (*)	0.24	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., CA	*DeLoach 00-25* (*)	0.25	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., NM	*DeLoach 00-26* (*)	0.26	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., NV	*DeLoach 00-27* (*)	0.27	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., NM	*DeLoach 00-38* (*)	0.38	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., TX	*DeLoach 00-41* (*)	0.41	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., TX	*DeLoach 00-42* (*)	0.42	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., TX	*DeLoach 00-43* (*)	0.43	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., CO	*Gaskin 103* (MO)	55	AF490798	AF484774
A	*Tamarix ramosissima* Ledeb.	NW: U.S., CO	*Gaskin 99* (MO)	59	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., TX	*Gaskin 41* (MO)	72	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., CA	*Gaskin 85* (MO)	77	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., CA	*Gaskin 88* (MO)	80	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., CA	*Gaskin 72* (MO)	87	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., NM	*Gaskin 50* (MO)	94	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., WA	*Gaskin 1204.1* (MO)	1204.1	AF490798	

Appendix 1. Continued.

trn S-*trn*G haplotype	Species	Origin	Collection #	DNA specimen #	GenBank accession *trn* S–*trn*G	GenBank accession ITS 1–2
A	*Tamarix ramosissima* Ledeb.	NW: U.S., OK	*Gaskin 1251* (MO)	1251.1	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., TX	*DeLoach 00-51* (*)	2009	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., TX	*DeLoach 00-46* (*)	2012	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., CO	*DeLoach 00-48* (*)	2013	AF490798	
A	*Tamarix ramosissima* Ledeb.	NW: U.S., WY	*DeLoach 00-49* (*)	2014	AF490798	
A	*Tamarix* cf. *ramosissima* Ledeb.	OW: Georgia	*Gaskin 753* (MO)	345	AF490798	
A	*Tamarix* cf. *ramosissima* Ledeb.	OW: Iran	*Gaskin 962* (MO)	962	AF490798	
A	*Tamarix* cf. *ramosissima* Ledeb.	OW: Iran	*Gaskin 964* (MO)	964	AF490798	
A	*Tamarix* cf. *ramosissima* Ledeb.	OW: Turkmenistan	*Gaskin 1087* (MO)	1087	AF490798	
A	*Tamarix* cf. *ramosissima* Ledeb.	OW: Turkmenistan	*Gaskin 1116* (MO)	1116	AF490798	
A	*Tamarix* cf. *ramosissima* Ledeb.	NW: U.S., TX	*DeLoach 00-12A* (*)	448	AF490798	
B	*Tamarix ramosissima* Ledeb.	OW: Kazakstan	*DeLoach s.n.* (*)	431	AF490776	AF484748
C	*Tamarix ramosissima* Ledeb.	OW: Kazakstan	*I. D. Mityaev 19* (*)	31	AF490796	
C	*Tamarix ramosissima* Ledeb.	NW: U.S., CA	*Gaskin 69* (MO)	84	AF490796	
D	*Tamarix ramosissima* Ledeb.	NW: U.S., AZ	*Gaskin 62* (MO)	110	AF539998	
E	*Tamarix ramosissima* Ledeb.	OW: Argentina	*Schulte 1* (MO)	449	AF490789	AF484761
E	*Tamarix ramosissima* Ledeb.	OW: Argentina	*Schulte 2* (MO)	454	AF490789	
F	*Tamarix ramosissima* Ledeb.	OW: Italy	*Gaskin 3065* (MO)	3065	AF490837	
G	*Tamarix ramosissima* Ledeb.	NW: U.S., KS	*Gaskin 105* (MO)	53	AF490782	
G	*Tamarix ramosissima* Ledeb.	NW: U.S., CO	*Gaskin 100* (MO)	57	AF490782	
G	*Tamarix ramosissima* Ledeb.	NW: U.S., CA	*Gaskin 70* (MO)	89	AF490782	
G	*Tamarix ramosissima* Ledeb.	NW: U.S., CA	*Gaskin 73* (MO)	90	AF490782	
G	*Tamarix ramosissima* Ledeb.	NW: U.S., OR	*Gaskin 1209* (MO)	1209	AF490782	
G	*Tamarix ramosissima* Ledeb.	NW: U.S., MO	*Gaskin 1253* (MO)	1253	AF490782	
	Tamarix gallica L.	OW: Spain	*Gaskin 3039* (MO)	3039		AF484807
	Tamarix canariensis Willd.	OW: France	*Gaskin 3049* (MO)	3049		AF484808
	Tamarix gallica L.	OW: France	*R. Sobhian 13* (*)	25		AF484775
	Tamarix canariensis Willd.	NW: U.S., LA	*Gaskin 36* (MO)	68		AF484802
	Tamarix canariensis Willd.	NW: U.S., LA	*Gaskin 34* (MO)	65		AF484801
	Tamarix canariensis Willd.	NW: U.S., LA	*DeLoach 3* (*)	24		AF484752
	Tamarix gallica L.	NW: U.S., TX	*DeLoach 00-15* (*)	0.15		AF484781
	Tamarix canariensis Willd.	NW: U.S., TX	*DeLoach 00-01* (*)	438		AF484778
	Tamarix canariensis Willd.	OW: Tunisia	*Kirk 2-Tunisia* (MO)	1276		AF484796
	Tamarix canariensis Willd.	OW: Spain	*Gaskin 3020* (MO)	3020		AF484806
	Myricaria alopecuroides Schrenk	OW: China	*Wang Jian Feng 10* (*)	18		AF484746